Effects of Lamb Age, Muscle Type, and 24-Hour Activity of Endogenous Proteinases on Postmortem Proteolysis[1,2]

G. Whipple and M. Koohmaraie

Roman L. Hruska U.S. Meat Animal Research Center, ARS, U.S. Department of Agriculture, Clay Center, NE 68933

ABSTRACT: The objectives of this study were to examine the amount of postmortem proteolysis in three different lamb muscles at different ages and to determine whether a relationship exists between the extent of myofibrillar degradation and certain endogenous proteinase activities. Wether lambs were slaughtered at 8 (n = 6) and 26 (n = 6) wk of age. Samples were taken from the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles for determining myofibrillar fragmentation index (MFI) at 1 and 7 d postmortem, cathepsins B and B + L, cystatin(s), μ- and m-calpain and calpastatin 24-h activities, and muscle fiber type and area. Muscle samples were removed for SDS-PAGE analysis at 0, 1, and 7 d postmortem. The SS muscle consisted of more ($P < .05$) oxidative fibers, whereas no age effects ($P > .10$) were observed for fiber type. The younger lambs had higher ($P < .01$) cathepsins B and B + L, cystatin, and calpastatin 24-h activities but less ($P < .01$) m-calpain activity than did the 26-wk-old lambs. Within each age, the SS muscle had the highest ($P < .05$) μ-calpain, m-calpain, and calpastatin specific activities. The MFI values and SDS-PAGE results indicate that less proteolysis occurred in the SS muscle. Samples from 26-wk-old lambs tended to have greater MFI values at 1 and 7 d postmortem, especially for the LM and GM muscles. From these results, it seems that less postmortem proteolysis occurs in younger and more oxidative muscles and this may be attributed to the greater calpastatin 24-h activity.

Key Words: Proteinases, Lambs, Proteolysis

J. Anim. Sci. 1992. 70:798–804

Introduction

Research has shown that the extent of postmortem proteolysis varies among different muscles (Olson et al., 1976; Koohmaraie et al., 1988) and in most cases is highly associated with meat tenderness. Myofibrillar proteolysis can be attributed to endogenous proteinase activity. Currently, two characterized proteolytic systems are known to hydrolyze myofibrillar proteins, the Ca^{2+}-dependent and catheptic proteinase systems (for review see Goll et al., 1983; Koohmaraie, 1988, 1992). Recent reports indicate that the Ca^{2+}-dependent (calpain) proteinase system may be more involved in postmortem myofibrillar proteolysis than the catheptic enzymes (Koohmaraie et al., 1988, 1991; Wheeler et al., 1990; Whipple et al., 1990a). However, frequently research designs consider only the longissimus muscle due to its economic importance, but it is not always representative of other muscles (Olson et al., 1977; Koohmaraie et al., 1988). In addition, proteinase activities vary with age and muscle (Whipple and Koohmaraie, 1991), which could be related to protein turnover rates (Goll et al., 1989). Therefore, the magnitude of postmortem proteolysis also may differ with age or muscle type. Objectives of this study were 1) to evaluate the effect of animal age and muscle type on postmortem proteolysis and 2) to examine the

[1]The authors would like to thank Sue Hauver, Kathy Mihm, Christi Christensen, and Ken Uptagraft for their laboratory assistance and Marilyn Bierman for typing this manuscript.

[2]Mention of a trade name, proprietary product, or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Received May 13, 1991.
Accepted October 3, 1991.

relationship between observed postmortem proteolysis and endogenous proteolytic systems.

Materials and Methods

Experimental Animals. Synthetic I (1/2 Finn, 1/4 Rambouillet, 1/4 Dorset) wether lambs were slaughtered at 8 (n = 6) and 26 (n = 6) wk of age weighing approximately 18 and 54 kg, respectively. Carcasses were stored at 4°C. At 24 h postmortem, the longissimus (**LM**, 2nd thoracic vertebra to 7th lumbar vertebra), gluteus medius (**GM**, middle gluteal), and supraspinatus (**SS**) muscles were removed from the left side of the carcass. The LM and SS muscles were selected to have a representation of a glycolytic muscle (LM) and an oxidative muscle (SS) according to Ouali and Talmant (1990).

Calpain and Calpastatin Activities. Twenty-four hours postmortem, activities of μ-calpain, m-calpain, and calpastatin were determined on a fresh, nonfrozen, 5-g sample from each of the three muscles according to the procedure described by Wheeler and Koohmaraie (1991) using 50 m*M* Tris-HCl, pH 8.3, instead of 50 m*M* sodium acetate, pH 5.8, as the extraction solution. Total (activity per gram of muscle) and specific (activity per milligram of extractable protein) caseinolytic activities were determined, because protein concentration differences were observed among the muscle samples. One unit of μ- and m-calpain activity was defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm in 1 h at 25°C. One unit of calpastatin activity was defined as the amount that inhibits 1.0 unit of DEAE-Sephacel® (Pharmacia, LKB Biotechnology, Piscataway, NJ)-purified m-calpain activity. Protein concentrations were determined using the biuret procedure (Gornall et al., 1949) after a trichloroacetic acid precipitation to remove interfering substances.

Cathepsins B and B + L Activities. Five grams of LM, GM, and SS muscle that had been frozen at 24 h postmortem and stored at −70°C were extracted to determine the activities of cathepsins B and B + L according to Method D of Koohmaraie and Kretchmar (1990). Protein concentrations were determined using a BCA protein assay reagent (Pierce, Rockford, IL). Total and specific activities were expressed as nanomoles of N-methylcourmarin (**NMec**) released · minute^{-1} · gram of muscle^{-1} and nanomoles of NMec released · minute^{-1} · milligram of extractable protein^{-1}, respectively. Cystatin(s) activity was defined as the ratio of cathepsins B + L total and specific activity after S-Carboxymethylated-papain-Sepharose affinity chromatography to cathepsins B + L total and specific activity before affinity chromatography.

Myofibril Fragmentation Index Measurements. Samples were excised from the LM, GM, and SS muscles at 24 h postmortem and were vacuum-packaged and stored for an additional 6 d at 4°C. Myofibril fragmentation indices (**MFI**) were determined on fresh muscle at 1 and 7 d postmortem according to the procedures of Culler et al. (1978). Biuret procedures were used to determine protein concentrations.

Muscle-Fiber Histochemistry and Fiber Area. At 24 h postmortem, samples were removed from the center of the LM, GM, and SS muscles, placed on cork perpendicular to fiber direction, frozen in isopentane cooled with liquid N_2, and stored at −70°C. Sections, 10-μm thick, were cut and allowed to air dry. After numerous trials and modifications using the procedure of Solomon and Dunn (1988), we were unable to clearly distinguish between βR and αR fibers in samples obtained from younger animals and in the older SS muscle. Therefore, because our purpose was to determine whether the muscles were predominantly oxidative or glycolytic, the acid incubation procedures of Guth and Samaha (1970) were used. Using a 10-min acid preincubation, pH 4.3, followed by a 45-min ATPase incubation, a clear distinction between oxidative and glycolytic fibers resulted. Those fibers that had moderate ATPase activity (intermediate in stain intensity) also were classified as oxidative fibers. These fibers possibly are the intermediate S-S fiber type classified in sheep by Suzuki and Cassens (1983). An average of 200 muscle fibers · muscle^{-1} · animal^{-1} were measured by Microcomp PM (Southern Micro Instruments, Atlanta, GA) interactive image analysis for planar morphometry.

Myofibril Isolation and SDS-PAGE. Myofibrils from LM, GM, and SS muscles were isolated according to the method of Goll et al. (1974) at 0 (within 30 min), 1, and 7 d postmortem. Protein concentrations were determined by biuret procedure. Electrophoretic (SDS-PAGE) procedures of Laemmli (1970) were followed with 75 μg of protein loaded per well. Myofibrillar proteins were separated using a discontinuous 7.5 to 15% acrylamide gradient slab gel with a 75:1 acrylamide to bisacrylamide ratio. For better resolution of desmin, 7.5% acrylamide gels were used. The acrylamide solution (30%) contained 50% glycerol.

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1985) for a split-plot design. The whole plot was animal age and the split plot was muscle. The whole plot error term was animal × age with animal being replicated. The split-plot error term was the residual error.

Results and Discussion

Difficulties were encountered in our attempts to classify βR, αR, and αW fibers for all muscles in both age groups using the procedure of Solomon and Dunn (1988). Suzuki and Cassens (1983) observed that in young sheep certain fiber types exist that are not present in adult sheep. This may have been partially the reason why difficulty was experienced. Therefore, we classified the fibers as either oxidative or glycolytic. With postnatal development, a change in muscle fiber metabolism occurs, such that the percentage of white or glycolytic fibers often increases (Ashmore et al., 1972), which agrees with our results (Table 1). The percentage of oxidative muscle fibers tended (P = .14) to be smaller in the older animals for all three muscles. In addition, a significant muscle effect was seen for muscle fiber type percentage. For both age groups, GM and LM muscles were very similar in their percentages of oxidative and glycolytic fibers. Oxidative fiber percentage was greater (P < .01) for the SS muscle, which agrees with the classification of Quali and Talmant (1990). Hunt and Hedrick (1977) reported that bovine LM and GM were similar in percentage of βR fibers and contained 46 and 53% αW fibers, respectively, which is similar to our glycolytic fiber percentage for these two muscles.

Muscle fiber area increased ($P \leq .01$) with age in both oxidative and glycolytic fibers. The LM muscle had the smallest (P < .05) muscle fibers and the SS muscle tended to have the largest (P = .06) muscle fibers at 26 wk of age. White et al. (1978) reported that fiber diameter increased in ovine quadricep muscles with age for both fiber types.

Olson et al. (1976) indicated that MFI values were good indicators of the extent of myofibrillar proteolysis that had occurred with postmortem storage and that differences exist among muscles. In our study, a significant age × muscle interaction was observed for d-1 MFI, whereas at d 7, muscle ($P \leq .01$) and age ($P \leq .10$) effects were present. Greater MFI values were seen for the LM and GM muscles of older lambs than for the SS muscle or younger lamb samples at both 1 and 7 d (Figure 1). By the larger MFI value, it seemed that the LM muscle from the 26-wk-old lambs responded more to postmortem proteolysis than the other two muscles. By 7 d postmortem, MFI values increased for all samples, but MFI values for the younger lambs tended (P = .10) to be smaller than those for the 26-wk-old lambs. Among muscles, the SS muscle had the lowest (P < .01) MFI values at 7 d postmortem for both ages. Results from SDS-PAGE did not confirm advanced proteolysis in the 26-wk-old LM muscle sample, which was indicated

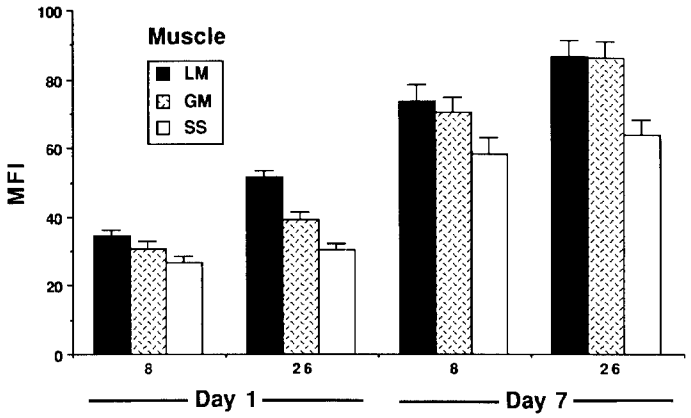

Figure 1. Myofibril fragmentation index values (MFI) at 1 and 7 d postmortem for the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles by lamb age. Age × muscle interaction (P < .01) for d-1 MFI values; muscle (P < .01) and age (P < .10) effects were present for d-7 MFI values.

by the d-1 MFI value. With 7 d of storage, a 30 to 32 kDa fragment(s) appeared and bands corresponding to troponin-T isoforms (Figures 2a and 3a) and desmin (Figures 2b and 3b) were degraded in the GM and LM muscles, for both age groups. However, in SS muscle samples of both age groups, the 30 to 32 kDa band(s) was less intense, and desmin and(or) troponin-T were faintly visible at d 7. Normally, in most muscles, troponin-T and desmin are hydrolyzed with postmortem aging (Koohmaraie et al., 1984). Koohmaraie (1990) infused $ZnCl_2$ into lamb carcasses, thus inhibiting myofibrillar proteolysis. No change in MFI values occurred with 14 d of postmortem storage, and desmin and troponin-T were not degraded in LM samples of that study. This inhibition also prevented postmortem tenderization, as suggested by Warner-Bratzler shear values. Although tenderness was not determined in this study, the lack of myofibrillar proteolysis indicated by MFI values and SDS-PAGE results suggests that the SS muscle would be least tender.

A significant age × muscle interaction was observed for cathepsins B + L total and specific activities, but no interaction effect existed for cathepsin B activities (Table 1). Younger lambs had higher (P < .01) cathepsins B + L total and specific activities than 26-wk-old lambs for all muscles. Cathepsins B and B + L specific activities were greater (P < .01) in the SS muscle of young lambs than in the other two muscles. In older lambs, cathepsin B specific activity was greater (P < .01) in the SS muscle than in the other two muscles, whereas no differences (P > .25) occurred in total activity among the muscles. Cathepsins B + L total activity was greatest (P <

Table 1. Least squares means for muscle fiber percentage and area and enzyme activities for the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles by animal age

		Age, wk						P-value		
		8			26					
Observation	SE	LM	GM	SS	LM	GM	SS	Age	Muscle	Age × muscle
Oxidative fibers										
%	4.3	58.6	51.5	78.5	52.7	49.2	70.2	.14	.01	.78
Area, μm^2	123	693	816	958	1,118	1,477	1,729	.01	.01	.41
Glycolytic fibers										
%	4.4	41.6	46.8	21.5	47.3	50.8	29.8	.12	.01	.89
Area, μm^2	191	958	1,128	1,446	1,324	2,027	2,559	.01	.01	.18
Overall fiber area, μm^2	137	761	942	1,049	1,208	1,765	1,973	.01	.01	.21
μ-Calpain										
Total activity[a]	.06	.35	.27	.58	.30	.42	.73	.13	.01	.16
Specific activity[b]	.01	.06	.05	.13	.05	.07	.18	.09	.01	.06
m-Calpain										
Total activity[a]	.07	1.23	1.12	1.27	1.62	1.60	1.83	.01	.05	.47
Specific activity[b]	.01	.22	.19	.29	.29	.29	.46	—	—	.01
Calpastatin										
Total activity[c]	.3	3.1	3.0	3.4	2.1	2.2	2.5	.01	.31	.97
Specific activity[d]	.05	.55	.53	.76	.38	.40	.64	.06	.01	.88
Cathepsins B + L										
Total activity[e]	5.3	194.7	191.7	188.2	110.0	75.4	81.6	—	—	.01
Specific activity[f]	1.4	34.3	32.6	42.5	19.7	13.6	20.6	—	—	.03
Cathepsin B										
Total activity[e]	5.7	79.6	93.5	94.9	66.4	64.0	73.3	.01	.18	.40
Specific activity[f]	1.3	14.1	16.2	21.4	11.8	11.9	18.0	.01	.01	.77
Cystatin(s) levels										
Total activity[g]	.2	2.7	2.4	1.9	1.8	1.5	1.2	.01	.01	.76
Specific activity[h]	.03	.48	.42	.43	.32	.27	.30	.01	.23	.92

[a]Caseinolytic activity/gram of muscle.
[b]Caseinolytic activity/milligram of extractable protein.
[c]Inhibition of casein hydrolysis by m-calpain/gram of muscle.
[d]Inhibition of casein hydrolysis by m-calpain/milligram of extractable protein.
[e]Nanomoles·minute^{-1}·gram of muscle^{-1}.
[f]Nanomoles·minute^{-1}·milligram of extractable protein^{-1}.
[g]Ratio of cathepsins B + L total activity after to before affinity chromatography.
[h]Ratio of cathepsins B + L specific activity after to before affinity chromatography.

.01) in the 26-wk-old LM muscle, whereas SS and GM muscles had similar activities. However, cathepsins B + L specific activity was least ($P < .01$) in 26-wk-old GM muscle, with no significant differences in activity between SS and LM muscles. Cystatin(s) levels based on total activities were least ($P < .05$) in young SS muscle. In 26-wk-old lambs, cystatin(s) levels did not differ significantly between SS and GM muscles. When specific activities were used in its determination, no differences ($P > .20$) among the muscles were detected, but levels were greater ($P < .05$) in 8-wk-old lambs. From these results, it seems that cathepsins B, B + L, and cystatin(s) activities cannot explain the differences in amount of postmortem myofibrillar proteolysis. In the SS muscle, less degradation occurred, whereas cathepsin specific activities tended to be greater and cystatin levels were the same as or less than those in the other muscles. Others also have reported no significant differences in cathepsins B and B + L activities when differences were observed in postmortem proteolysis (Wheeler et al., 1990; Whipple et al., 1990a). These enzymes are normally located in lysosomes and presumably

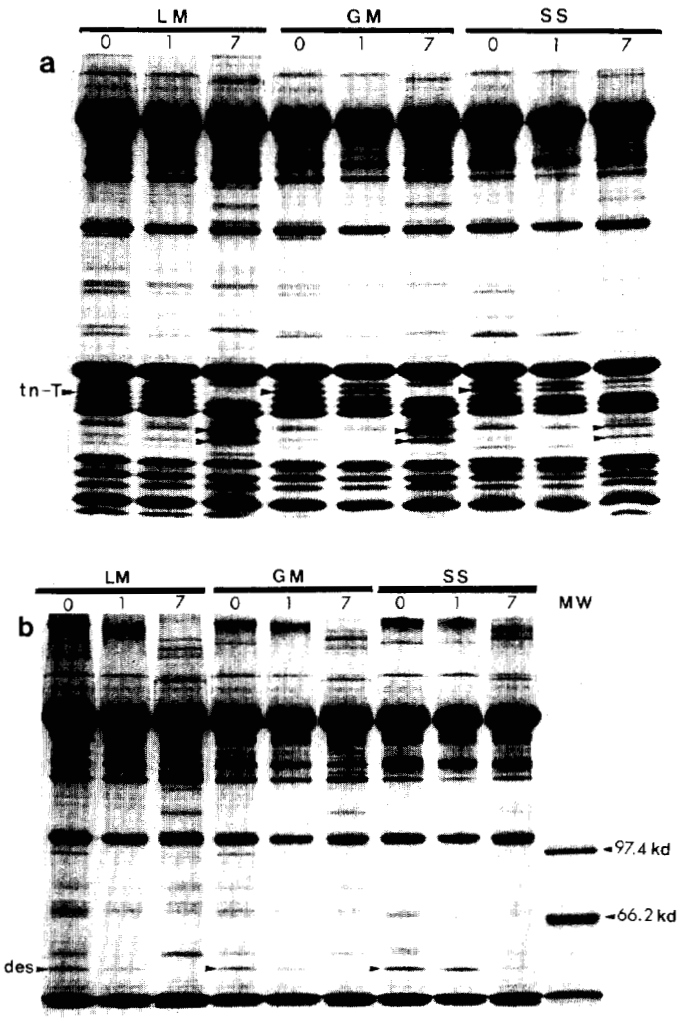

Figure 2. (a) The SDS-PAGE (7.5 to 15%) of purified myofibrils taken from the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles of 26-wk-old lambs at 0, 1, and 7 d postmortem. (b) The SDS-PAGE (7.5%) of purified myofibrils taken from the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles of 26-wk-old lambs at 0, 1, and 7 d postmortem.

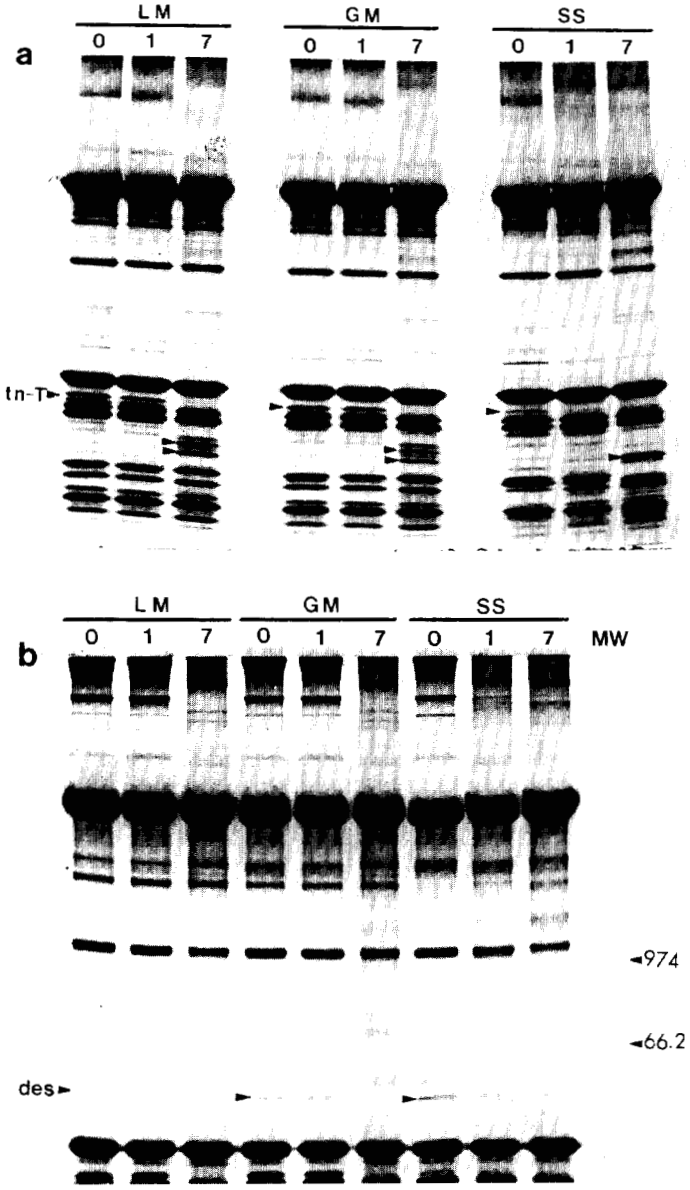

Figure 3. (a) The SDS-PAGE (7.5 to 15%) of purified myofibrils taken from the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles of 8-wk-old lambs at 0, 1, and 7 d postmortem. (b) The SDS-PAGE (7.5%) of purified myofibrils taken from the longissimus (LM), gluteus medius (GM), and supraspinatus (SS) muscles of 8-wk-old lambs at 0, 1, and 7 d postmortem.

have to be released to have access to myofibrils. It has been assumed that during postmortem storage lysosomes rupture, thereby releasing the cathepsins into the cytosol. However, the only experiment conducted to test the accuracy of this assumption failed to indicate lysosomal rupture after 28 d of postmortem storage (LaCourt et al., 1986). In addition, myosin and actin are degraded by cathepsins (Ouali et al., 1987). However, Bandman and Zdanis (1988) did not detect myosin degradation in muscle samples aged 28 d under normal cooler conditions. Neither was myosin or actin degradation apparent in the present study (Figures 2a and 3a).

The other proteolytic mechanism thought to be involved in postmortem proteolysis is the calpain proteinase system, and differences in activity are known to exist among different muscle types (Olson et al., 1977; Koohmaraie et al., 1988; Ouali, 1990). In the present study, significantly greater m-calpain total and specific 24-h activities were observed in 26-wk-old lambs within muscle. Specific m-calpain activity was greatest in the SS muscle for both age groups, whereas no differences in either specific or total activity occurred between LM and GM muscles. These observations in relation to the extent of postmortem proteolysis are probably not relevant, because m-calpain is not active under normal postmortem conditions (Vidalenc et al., 1983; Ducastaing et al., 1985; Koohmaraie et al., 1987). On the other hand, μ-calpain is active postmortem, and its activity is responsible in part for postmortem myofibrillar proteolysis (for review, see Koohmaraie, 1988, 1992). In the present study, total and specific 24-h activities of μ-calpain were greater ($P < .01$) in the SS muscle than in the other muscles for both age groups (Table 1). Therefore, μ-calpain 24-h activity seems to have a negative relationship with the amount of proteolysis that occurred in the SS muscle. However, the SS muscle also had greater ($P < .01$) calpastatin 24-h specific activity than either of the other two muscles at both age periods, which agrees with data of Ouali and Talmant (1990). In addition, younger lambs had more ($P < .05$) calpastatin 24-h activity than did 26-wk-old lambs within each muscle. The greater calpastatin activity in the SS muscle could have decreased the amount of myofibrillar protein hydrolysis by μ-calpain. Others also have reported a relationship between calpastatin 24-h activity and amount of postmortem proteolysis (Wheeler et al., 1990; Whipple et al., 1990a; Koohmaraie et al., 1991).

In addition to calpastatin, other mechanisms or regulators, such as a calpain activator (Pontremoli et al., 1990), could be involved in the regulation of the calpain proteolytic system in postmortem muscle. Also, endogenous differences between muscle types (red vs white) could modify these regulatory elements postmortem, because the rate of muscle protein turnover differs between these muscle types (Watt et al., 1982; Lewis et al., 1984). For example, zinc (an inhibitor of cysteine proteinases) concentration is higher in red muscles (Kondo et al., 1991) and has a detrimental effect on postmortem proteolysis and tenderness (Koohmaraie, 1990). Therefore, zinc concentration could be one factor contributing to the differences in proteolysis among different muscle types. However, Whipple et al. (1990a) failed to find any differences in muscle fiber type and area, muscle pH, and water-soluble free calcium and zinc contents between LM muscles from *Bos taurus* and *Bos indicus* breeds of cattle, even though differences were observed in postmortem myofibrillar proteolysis, tenderness, and calpastatin 24-h activity. On the other hand, Whipple et al. (1990b) indicated that although calpastatin 24-h activity may serve as a predictor of tenderness ($r^2 = .44$), either percentage ($r^2 = .16$) or area ($r^2 = .13$) of red muscle fibers also was able to explain some variation in LM tenderness. Therefore, the possibility exists that factors associated with muscle type may regulate in part postmortem myofibrillar proteolysis by way of the calpain system or some other mechanism.

Implications

Postmortem proteolysis seemed less extensive in more oxidative muscles than in more glycolytic muscles, as shown in 8-wk-old lambs and in the supraspinatus muscle of 26-wk-old lambs. This difference in degradation possibly could be related to the calpain proteinase system. Calpastatin may regulate the activity of μ-calpain, thus preventing the degradation of myofibrillar proteins. Extent of postmortem proteolysis in different muscle types and animals varying in age may be related to the mechanisms involved in muscle protein turnover in the live animal.

Literature Cited

Ashmore, C. R., G. Tompkins, and L. Doerr. 1972. Postnatal development of muscle fiber types in domestic animals. J. Anim. Sci. 34:37.

Bandman, E., and D. Zdanis. 1988. An immunological method to assess protein degradation in post-mortem muscle. Meat Sci. 22:1.

Culler, R. D., F. C. Parrish, Jr., G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food. Sci. 43:1177.

Ducastaing, A., C. Valin, J. Schollmeyer, and R. Cross. 1985.

Effects of electrical stimulation on postmortem changes in the activities of two calcium dependent neutral proteinases and their inhibitor in beef muscle. Meat Sci. 15:193.

Goll, D. E., W. C. Kleese, and A. Szpacenko. 1989. Skeletal muscle proteases and protein turnover. In: D. R. Campion, G. J. Hausman, and R. J. Martin (Ed.) Animal Growth Regulation. pp 141–182. Plenum Publishing, New York.

Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Shannon, S. K. Sathe, and M. Muguruma. 1983. Role of muscle proteases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137.

Goll, D. E., R. B. Young, and M. H. Stromer. 1974. Separation of subcellular organelles by differential and Z-line degradation in postmortem bovine, porcine and rabbit muscle. Proc. Rec. Meat Conf. 27:266.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum-protein by means of the biuret reaction. J. Biol. Chem. 177:751.

Guth, L., and F. A. Samaha. 1970. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. 28: 365.

Hunt, M. C., and H. B Hedrick. 1977. Profile of fiber types and related properties of five bovine muscles. J. Food Sci. 42: 513.

Kondo, H., M. Kimura, and Y. Itokawa. 1991. Manganese, copper, zinc and from concentrations and subcellular distribution in two types of skeletal muscle. Proc. Soc. Exp. Biol. Med. 196:83.

Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Proc. Recip. Meat Conf. 41:89.

Koohmaraie, M. 1990. Inhibition of postmortem tenderization in ovine carcasses through infusion of zinc. J. Anim. Sci. 68: 1476.

Koohmaraie, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in postmortem proteolysis and meat tenderness. Biochimie (In press).

Koohmaraie, M., W. H. Kennick, E. A. Elgasim, and A. F. Anglemier. 1984. Effects of postmortem storage on muscle protein degradation: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:292.

Koohmaraie, M., and D. H. Kretchmar. 1990. Comparisons of four methods for quantification of lysosomal cysteine proteinase activities. J. Anim. Sci. 68:2362.

Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson, and A. S. Babiker. 1988. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407.

Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson, and J. D. Crouse. 1987. Effect of postmortem storage on calcium-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19:187.

Koohmaraie, M., G. Whipple, D. H. Kretchmar, J. D. Crouse, and H. J. Mersmann. 1991. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69:617.

LaCourt, A., A. Obled, C. DeVal, A. Ouali, and C. Valin. 1986. Postmortem localization of lysosomal peptide hydrolase, cathepsin B. In: V. Turk (Ed.) Cysteine Proteinases and Their Inhibitors. p 239. Walter de Gruyter, New York.

Laemmli, U. K. 1970. Cleavage of storage proteins during the assembly of the head bacteriophage T4. Nature 227:680.

Lewis, E. M., F. J. Kelly, and D. F. Goldsprink. 1984. Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. Biochem. J. 217:517.

Olson, D. G., F. C. Parrish, Jr., W. R. Dayton, and D. E. Goll. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42:117.

Olson, D. G., F. C. Parrish, Jr., and M. H. Stromer. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sci. 41:1036.

Ouali, A. 1990. Meat tenderization: Possible causes and mechanisms. A review. J. Muscle Food 1:129.

Ouali, A., N. Garrel, A. Obled, C. Deval, and C. Valin. 1987. Comparative action of cathepsins D, B, H, L and of a new lysosomal cysteine proteinase on rabbit myofibrils. Meat Sci. 19:83.

Ouali, A., and A. Talmant. 1990. Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. Meat Sci. 28:331.

Pontremoli, S., P. L. Viotti, M. Michetti, B. Sparatore, F. Salamino, and E. Melloni. 1990 Identification of an endogenous activator of calpain in rat skeletal muscle. Biochem. Biophys. Res. Comm. 171:569.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Solomon, M. B., and M. C. Dunn. 1988. Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine and porcine skeletal muscle. J. Anim. Sci. 66: 255.

Suzuki, A., and R. G. Cassens. 1983. A histochemical study of myofiber types in the serratus ventralis thoracis muscle of sheep during growth. J. Anim. Sci. 56:1447.

Vidalenc, P., P. Cottin, N Merdaci, and A. Ducastaing. 1983. Stability of two Ca^{2+}-dependent neutral proteinases and their specific inhibitor during post-mortem storage of rabbit skeletal muscle. J. Sci. Food Agric. 34:1241.

Watt, P. W., F. J. Kelly, D. G. Goldspink, and G. Goldspink. 1982. Exercise-induced morphological and biochemical changes in skeletal muscles of the rat. J. Appl. Physiol. 53:1144.

Wheeler, T. L., and M. Koohmaraie. 1991. A modified procedure for simultaneous extraction and subsequent assay of calcium-dependent and lysosomal protease systems from a skeletal muscle biopsy. J. Anim. Sci. 69:1559.

Wheeler, T. L., J. W. Savell, H. R. Cross, D. K. Lunt, and S. B. Smith. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. J. Anim. Sci. 68:4206.

Whipple, G., and M. Koohmaraie. 1991. Effects of animal age on endogenous proteinase activities and their relationship with muscle growth in three different ovine muscles. J. Anim. Sci. 69(Suppl. 1):336 (Abstr.).

Whipple, G., M. Koohmaraie, M. E. Dikeman, and J. D. Crouse. 1990b. Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. J. Anim. Sci. 68: 4193.

Whipple, G., M. Koohmaraie, M. E. Dikeman, J. D. Crouse, M. C. Hunt, and R. D. Klemm. 1990a. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. J. Anim. Sci. 68:2716.

White, N. A., II, M. D. McGavin, and J. E. Smith. 1978. Age-related changes in percentage of fiber types and mean fiber diameters of the ovine quadriceps muscles. Am. J. Vet. Res. 39:1297.